Invention Journal of Research Technology in Engineering & Management (IJRTEM)
ISSN: 2455-3689
www.ijrtem.com Volume 2 Issue 8 ǁ August 2018 ǁ PP 23-31

Survivin Immunoreactivity in the Gastric Mucosa of Rats Feedind with Carpet Shell Clam (*Ruditapes decussatus*)

[1,]Aslı Muratlı, [2,]Mehmet Rıza Gezen
[1,]*Department of Pathology, Zübeyde Hanım Application and Research Center, Başkent University, İzmir/Turkey*
[2,]*Vocational College of Healt Sciences, Çanakkale Onsekiz Mart University, Çanakkale/Turkey*

ABSTRACT : *Survivin has been studied many times because of its overexpression in several types of cancer including lung, kidney, skin, endometrium, stomach, colon, breast, prostate, over, hematologic, head and neck cancers, histopathology features and polymorphisms in the promoter region which belongs to the inhibitör of apoptosis gene family by researchers. There is no study of survivin immunoreactivity in the gastric mucosa of the rats fed with carpet shell clam grown in the Dardanelles. In this study, it was aimed to investigate the effects of carpet shell clam fed rats on survivin production in the gastric mucosa. The carpet shell clam given as food to the rats were removed from the Dardanelles Çardak region. Four groups of rats are included in the study, group 1 (n=6), control group fed with standard rat food, group 2 (n=6), 75% carpet shell clam and 25% standard rat food daily, group 3 (n=6), 75% carpet shell clam and 25% standard rat food every two days, group 4 (n=6), 75% carpet shell clam and 25% standard rat food every three days. To detect survivin localization in the tissues, the LAB-SA Detection System was used. Survivin immunoreactivity was detected of epithelial cells in the gastric mucosa of rats fed with carpet shell clam. After the immunohistochemical staining processing all gastric tissue samples are evaluated in terms of survivin immunoreactivity with light microscopy and image analysis software. Survivin immunoreactivity was detected 0% in the first group, 83.33% in the second group, 61.83% in the third group and 32.67% in the fourth group. There was statistically significant difference between the survivin immunoreactivity in the gastric gland cells of the rats in the experimental and control groups (p> 0.05). Survivin production in the gastric mucosa of rats suggests that consumption of carpet shell clam may cause tissue damage.*

KEY WORDS : *Dardanelles, immunohistochemistry, stomach, carpet shell clam, survivin*

I. INTRODUCTION

The association between exposure to heavy metals, either occupational or environmental, and cancer has been extensively studied [1, 2]. The mechanisms involved in heavy metal induced carcinogenicity seem to be diverse and are far from being completely understood [3]. Heavy metals are natural elements of the earth's crust. Although not metabolized, some heavy metals participate in some essential metabolic activities: for example, copper (Cu) is essential for the synthesis of hemoglobin and cobalt (Co) is involved in erythropoiesis. However, if the amount of these essential elements exceeds normal levels, they can become toxic and cause serious health risks [4]. Prolonged exposure to mercury (Hg), lead (Pb), chromium (Cr), cadmium (Cd), arsenic (As), copper (Cu), vanadium (V), nickel (Ni) and zinc (Zn) can cause deleterious health effects in humans, namely, chronic inflammatory conditions and a higher risk for several cancers, cardiac, pulmonary and neurological diseases [5-7]. This scenario has been aggravated by erroneous human intervention that has significantly changed their natural cycle and balance, causing abnormal accumulation and environmental pollution. [5-7]. Concerning renal cell carcinoma, several heavy metals have been implicated as risk factors, namely Cd [8] and Pb [9]. Nevertheless, this causal association has not been definitively established yet [10].

In our previous research have investigated the accumulation of heavy metals in the carpet shell clam, sea snails and oysters from the Dardanelles Umurbey region. In this research, Zn in carpet shell clams, Zn and Mn in clams, Zn in oysters, Al, Zn, Fe, Cu and Mn in sea snails found the metals as high. If the same zone is in seawater, the Zn level is high [11]. In sea chestnuts growing in Dardanells, the values of Al, Zn, and Fe in samples taken from Gelibolu Hamzakoy station are high. Al and Fe values were higher in samples taken from Çardak region. Al, Fe and Zn values were higher in samples taken from Umurbey region. Al, Fe and Zn values were higher in samples taken from Çamburnu region [12]. In our previous research have investigated the accumulation of heavy metals in the carpet shell clam, clam, sea snails and oysters from the Dardanelles Karacaören region. In this research, Al, Zn and Fe in carpet shell clams, Zn and Mn in clams, Zn in oysters, Al, Zn, Fe, Cu and Mn in sea snails found the metals as high [13]. Survivin is a member of the inhibitor of apoptosis protein family, and is thought to inhibit apoptosis and regulate mitosis. It has unique roperties, with bifunctional roles as a cell-cycle regülator and an apoptosis inhibitör [14].

Although survivin is strongly expressed in embryonic and fetal organs, it is undetectable in most terminally differentiated normal adult tissues [15]. However, survivin is overexpressed in various cancers, including lung, breast, colon, stomach, esophageal, pancreatic, bladder, uterine, and ovarian cancers; large-cell non-Hodgkin's lymphoma; leukemia; neuroblastoma; melanoma; and nonmelanoma skin cancer, compared with its expression in normal tissues [16]. The lumen-facing surface of the stomach is covered with a single-layered prismatic epithelium, and surface epithelial cells produce mucus. The lamina propria is also branched in the mucosa, with tubular gastric glands. Gastric glands consist of three parts: isthmus, neck and base. There are parietal cells and stem cells in the istmus region. There are parietal cells, stem cells and mucous neck cells in the neck. In the basale region, there are parietal, basal and enteroendocrine cells. Mucous neck cells secrete mucus. Parietal cells secrete hydrochloric acid. Chief (zymogen) cells secrete pepsinogen and lipase enzyme. Enteroendocrine cells produce serotonin, glucagon, vasoactive intestinal polypeptide and somatostatin [17]. Because of the critical role of survivin in carcinogenesis [15], we evaluated the correlation between carpet shell clam-fed rats and survivin release.

II. MATERIAL AND METHOD

Animal Model *:* The rats were kept for 30 days under appropriate conditions of temperature/humidity and a 12-h light cycle while being provided sufficient water and feed. The rats were randomly selected and divided into 4 groups. For the first study group (n: 6), was the control group; standard rat diet was given every days. For the second study group (n: 6), 75% carpet shell clam + 25% standard rat diet standard rat feeds were given daily. For the third study group (n: 6), 75% carpet shell clam + 25% standard rat diet was given every two days. Standard rat diet was given the other day. For the fourth group (n: 6), 75% carpet shell clam + 25% standard rat diet was given every three days. Standard rat diet was given the other two day. Rats were fed twice daily for 30 days at 15% of their weight every morning and evening at the same time. The carpet shell clam given as food to the rats were removed from the Dardanelles Çardak region (Fig. 1).

Figure 1. *The area where the carpet shell clam are collected.* ***Arrow:*** *Çardak Lagoon (Çanakkale, Turkey),* ***Star:*** *Dardanelles*

Average 40-60 g weight were selected. After the beaks were overcooked, the meat broke off and the meat at 100 degrees was dried. It was weighed into each rat's weight and 10 mg/kg intraperitoenal ketamine hydrochloride (Ketalar, Eczacibasi, Istanbul, Turkey), and 20 mg/kg of xylazine 2% (Rompun, Bayer Turkey Pharmaceutical Ltd., Istanbul, Turkey) were anesthetized. The rats were anesthetized and then sacrificed.

Histological evaluation : The stomach tissues were maintained in immunofix (Leica) for 24 hours for histopathological examination. The tissue was routinely subjected to histopathological procedures and blocked. Immunohistochemical staining method was applied by cutting the paraffin embedded stomach tissues 3 microns in thickness. The LAB-SA Detection System, (Histostain-Plus Bulk Kit, Invitrogen) was applied to determine immunohistochemical localization of survivin in tissues. Slides were incubated with polyclonal rabbit anti-survivin (Diagnostic BioSystems) antibody, which was diluted 1: 500 for the stomach tissue, for an hour at room temperature, in the humidity chamber. Diaminobenzidine-tetra hydrochloride (DAB, Invitrogen Corporation) was used as the colorant. Also hematoxylin stain was used as a nuclear counterstain. Dye samples were evaluated on the Zeiss AXIO Scope 1 brand research microscope. Analysis of survivin immunoreactive cells in the stomach

tissue was performed using the Leica LAS V3.8 image analysis system. Five of the sections from the blocks containing the stomach tissues of all the rats in all groups were stained. From the stained sections, 1000 cells were counted and immunoreactive cells were identified among these cells. For this purpose;
Immunopositive cells / Total cell count (1000) ------- X 100 % = ------- % formula were used [18-19].

Statistical analysis : Data was analyzed using SPSS program, version 19.0 One-way analysis of variance (ANOVA), Tukey's test was used to analyze the data. The difference between the groups was considered significant in the results of $p < 0.05$.

III. RESULTS

In immunohistochemical staining with survivin, a significant difference was observed in the gastric gland cells of the rats given carpet shell clam per day, every other day and every three days compared to rats fed with normal feed ($p<0.05$). Dark brown staining in the cytoplasm of the cells was considered positive. Survivin immunoreactivity could not be detected in gastric mucosa cells of rats fed standard rat diet (Fig. 2).

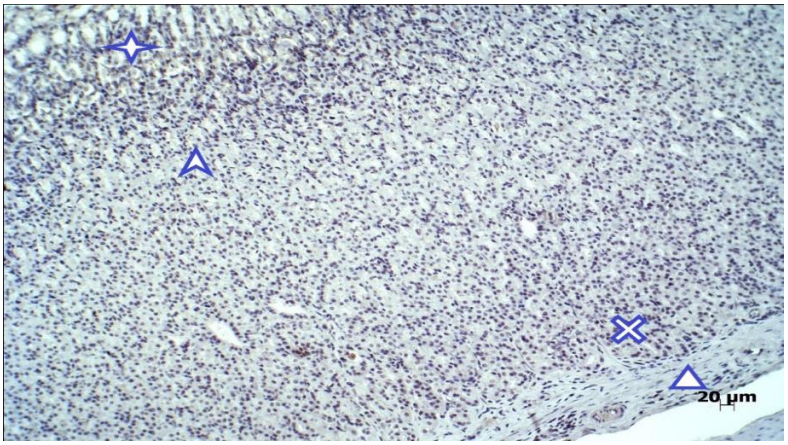

Figure 2. For the first study group was the control group; Rat stomach mucosa, (Survivin, Bar=20 µm). **Star:** Surface epithelial region, **Arrow head:** Neck region, **Crossed:** Basale region, **Pointed arrow**: Muscle mucosa
In immunohistochemical staining of gastric mucosa, basal, neck and surface epithelial cell cytoplasms were positively stained with survivin. There was no significant difference in survivin staining intensity between cells. 84.33% of superficial epithelial cells, 82.5% of neck cells and 83.83% of basal cells were positive for survivin (Fig. 3).

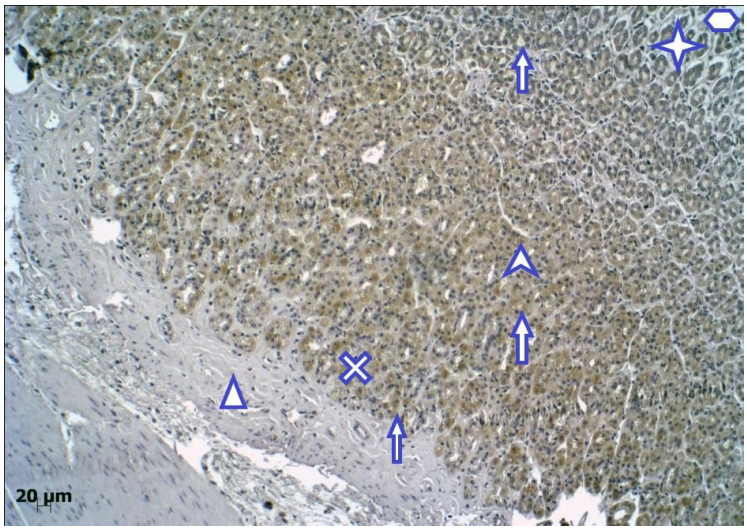

Figure 3. For the second study group; Rat stomach mucosa, (Survivin, Bar=20 µm). **Hexagon:** Gastric lumen; **Star:** Surface epithelium cells; **Arrow head:** Neck region; **Crossed:** Basale region; **Pointed arrow:** Muscle mucosa, **Arrows:** Survivin enzyme positive cells

In immunohistochemical staining of gastric mucosa, basal, neck and surface epithelial cell cytoplasms were positively stained with survivin. There was no significant difference in survivin staining intensity between cells. 63.67% of superficial epithelial cells, 58.17% of neck cells and 62,17% of basal cells were positive for survivin (Fig. 4).

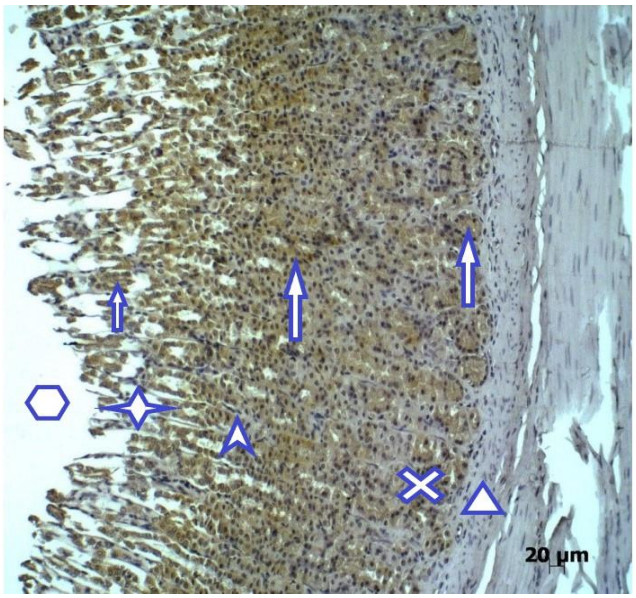

Figure 4. For the third study group; Rat stomach mucosa, (Survivin, Bar=20 µm). **Hexagon:** Gastric lumen; **Star:** Surface epithelium cells; **Arrow head:** Neck region; **Crossed:** Basale region; **Pointed arrow:** Muscle mucosa, **Arrows:** Survivin enzyme positive cells In immunohistochemical staining of gastric mucosa, basal, neck and surface epithelial cell cytoplasms were positively stained with survivin. The cytoplasm of basal cells was more intensely stained than surface epithelial cells and neck cells. 20.83% of superficial epithelial cells, 25% of neck cells and 35.83% of basal cells were positive for survivin (Fig. 5).

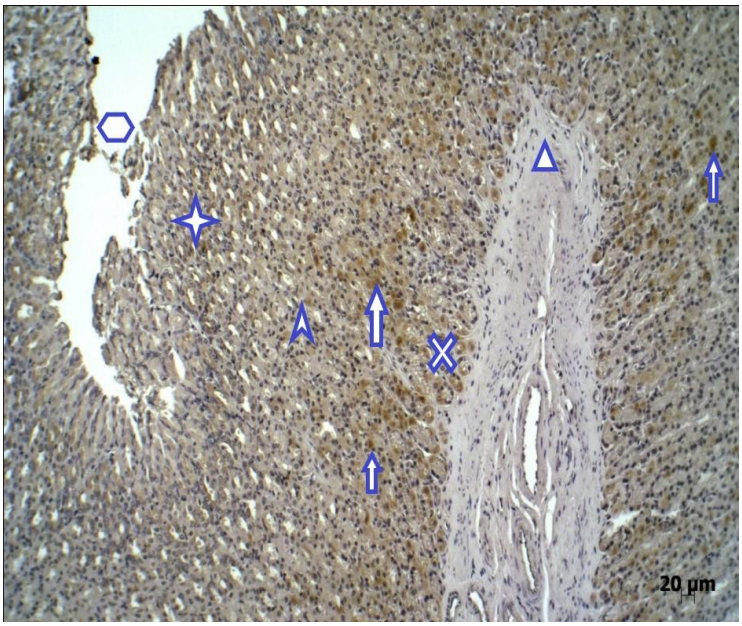

Figure 5. For the fourth group; Rat stomach mucosa, (Survivin, Bar=20 µm). **Hexagon:** Gastric lumen; **Star:** Surface epithelium cells; **Arrow head:** Neck region; **Crossed:** Basale region; **Pointed arrow:** Muscle mucosa,

Arrows: Survivin enzyme positive cells
In the epithelial cells of the gastric mucosa in the all group, survivin immunopositive cells could not be detected by negative staining (Fig. 6).

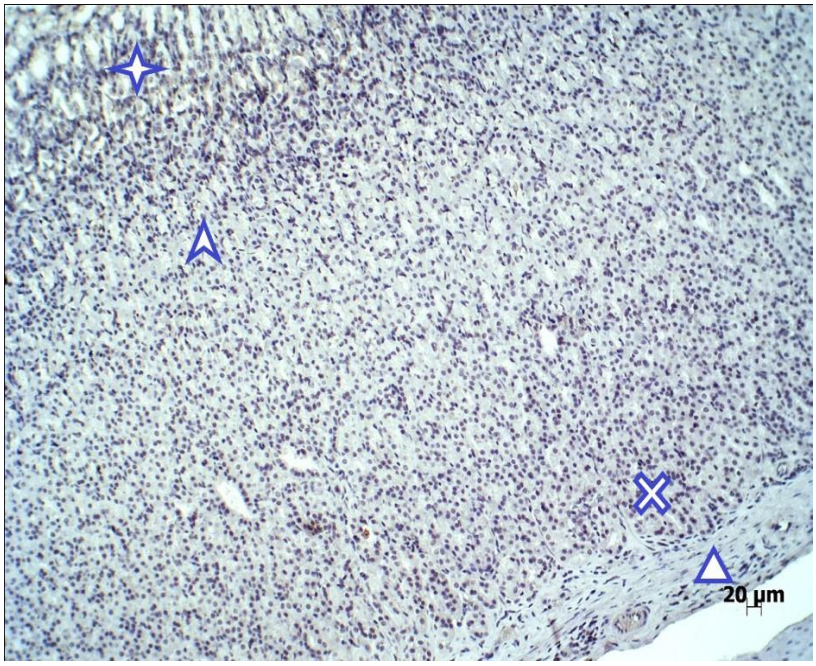

Figure 6. For the second study group; Rat stomach mucosa, negative control, (Survivin, Bar=20 µm). **Star:** Surface epithelium cells; **Arrow head:** Neck region; **Crossed:** Basale region; **Pointed arrow:** Muscle mucosa,
The increase of survivin immunoreactivity in the gastric mucosa cells between the control group and the other groups was statistically significant. A statistically significant difference was also found among the groups in the gastric mucosa cells of the rats fed carpet shell clam. 83.33% of the second group, 61.83% of the third group and 32.67% of the fourth group were positive for survivin (Fig. 7).

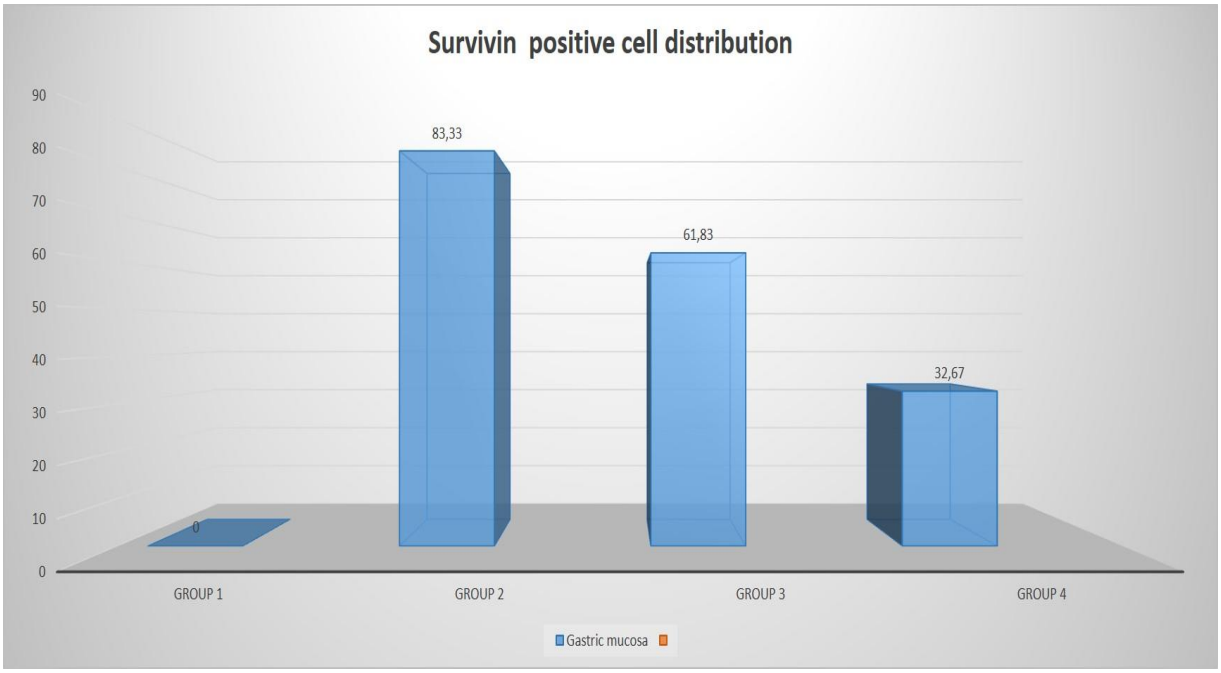

Figure 7. Distribution of survivin immunoreactivity total in gastric mucosa cells between groups
The increase of survivin immunoreactivity in the superficial epithelium cells between the control group and the other groups was statistically significant. A statistically significant difference was also found among the groups in the superficial epithelium cells of the rats fed carpet shell clam. 84.33% of the second group, 63.67% of the third group and 20.83% of the fourth group were positive for survivin (Fig. 8).

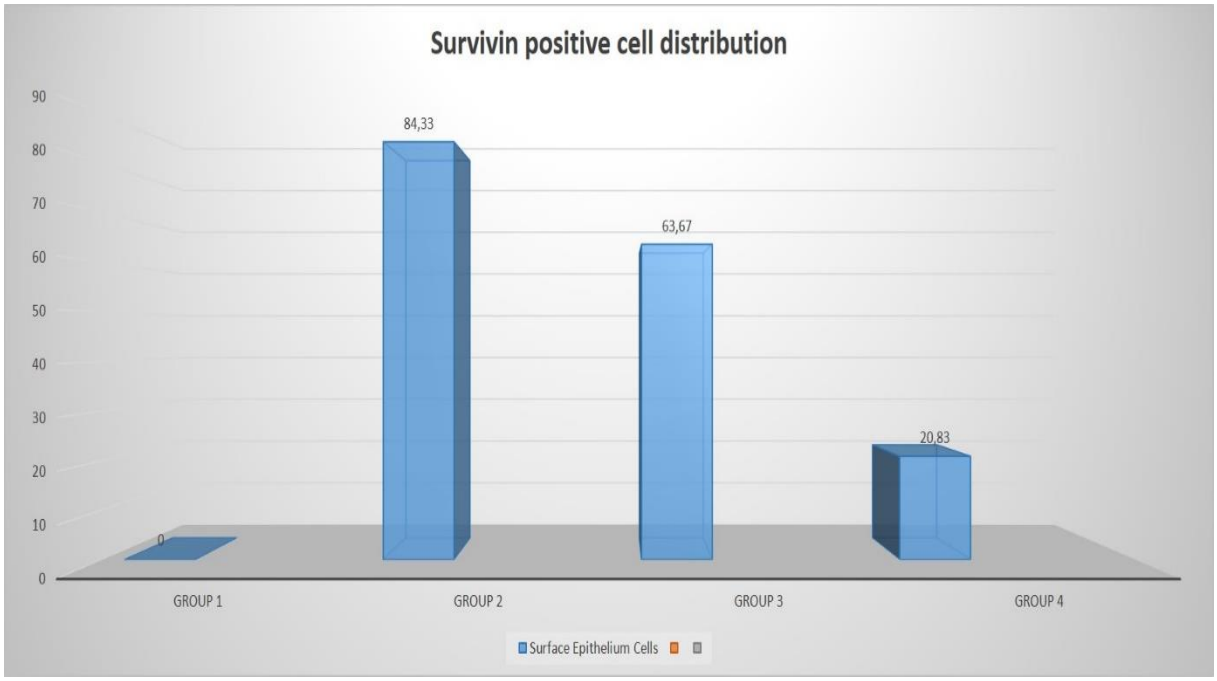

Figure 8. Distribution of survivin immunoreactivity in surface epithelium cells between groups

The increase of survivin immunoreactivity in the neck cells between the control group and the other groups was statistically significant. A statistically significant difference was also found among the groups in the neck cells of the rats fed carpet shell clam. 82.5% of the second group, 58.17% of the third group and 25% of the fourth group were positive for survivin (Fig. 9).

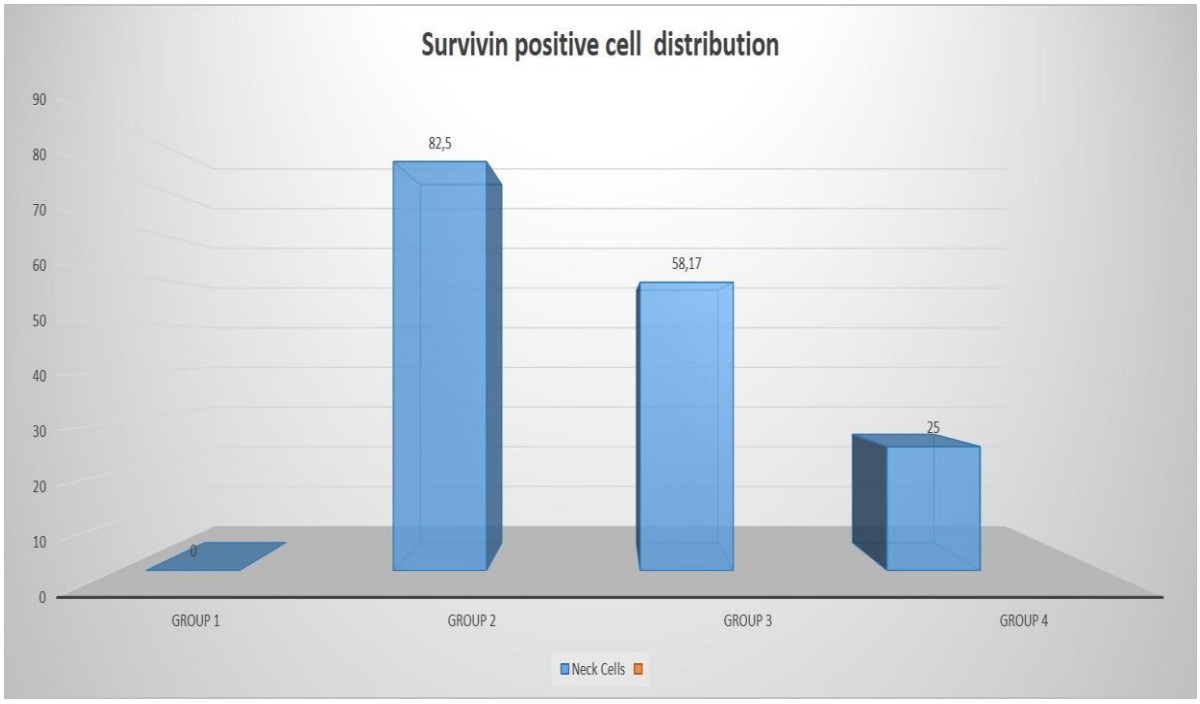

Figure 9. Distribution of survivin immunoreactivity in isthmus and neck regio cells between groups

The increase of survivin immunoreactivity in the basale cells between the control group and the other groups was statistically significant. A statistically significant difference was also found among the groups in the basale cells of the rats fed carpet shell clam. 83.83% of the second group, 62.17% of the third group and 35.83% of the fourth group were positive for survivin (Fig. 10).

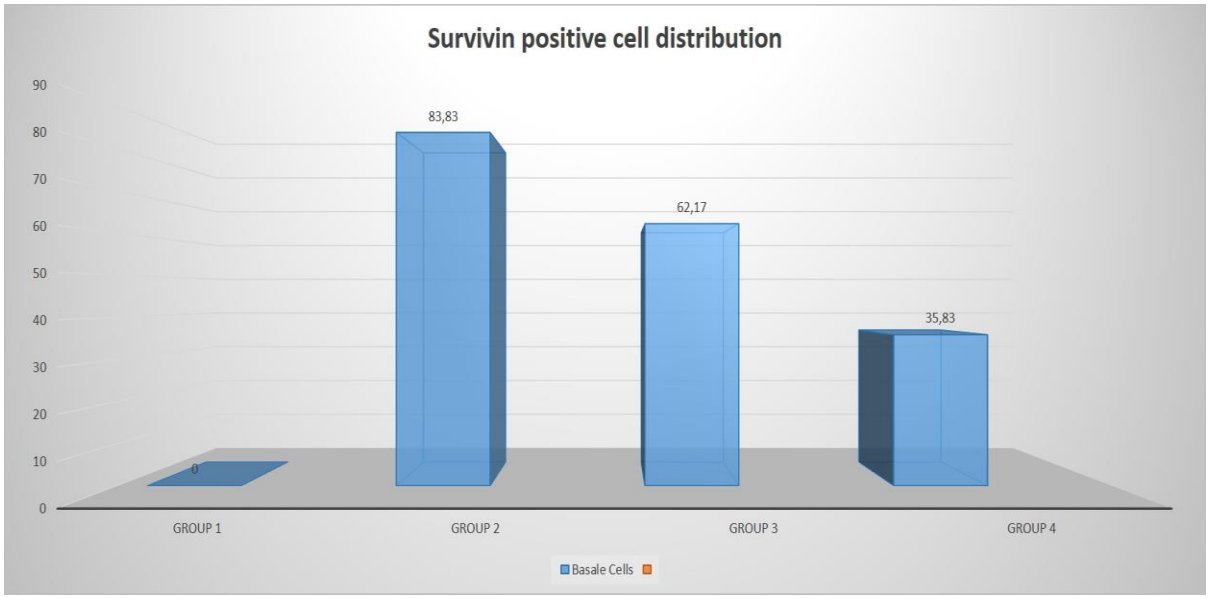

Figure 10. *Distribution of survivin immunoreactivity in basale regio cells between groups*

IV. DISCUSSION

The aim of this study was to determine survivin release in the gastric mucosa of rats fed with carpet shell clam, using immunohistochemical technique. We found survivin release in gastric mucosa samples, but not in the adjacent normal gastric mucosa epithelium cells. Survivin immunoreactive cells were found in 83.83% of the gastric mucosa cells of rats fed with carpet shell clam every day. Survivin immunoreactive cells were found in 61.83% of the gastric epithelial cells of rats fed with carpet shell clam every other day. Survivin immunoreactive cells were found in 32.67% of the gastric epithelial cells of rats fed with carpet shell clam every three days There is a lot of research on survivin production, but there is no research on survivin release in stomach tissues of carpet shell clam fed rats. In our study, we found an increase in survival in the stomach, but we could not find any cancer in the stomach. We had chronic inflammation in the preliminary study of this study. Our findings regarding survivin release as causing chronic inflammation are similar to other investigators.

Environmental factors have been considered responsible for at least 80% of the incidence of neoplastic diseases [20]. It is well known that heavy metals, in particular Cd, have an oncogenic role in tumourigenesis [21]. Cd was identified as a human carcinogen, being associated with lung cancer after occupational exposure. In addition, it has been implicated in kidney, breast and prostate cancers [22]. In fact, there is strong evidence of chronic inflammation induced by heavy metals as one of the main underlying mechanisms [21]. Survivin is an antiapoptotic protein belonging to the inhibitor of apoptosis protein family. It is a bifunctional protein that regulates cell division and suppresses apoptosis. Survivin is highly expressed in various human malignancies, but its expression is very low or below the level of detection in normal adult tissues [23, 24]. Survivin is usually expressed in the G2 /M phase of the cell cycle, when it is thought to disrupt the apoptosis signaling pathways and to promote the survival of abnormal cells, offering a significant advantage to tumor cells overexpressing survivin [25]. The elevated expression of survivin is thought to be a negative prognostic factor for tumors and its overexpression is reported to be associated with shortened survival [14]. Many studies have also shown that tumors expressing survivin are resistant to the apoptosis that is induced by anticancer drugs [26, 27]. Targeting of survivin using adenoviral antisense vectors was reported to enhance the sensitivity of tumor cells to chemotherapy and radiotherapy [28]. Clarification of the survivin signaling pathway will provide new predictive and prognostic information for cancer diagnosis and could lead to the development of new therapeutic alternatives for a variety of cancers [29, 30].High survivin expression has been detected in several cancer types in humans, including colorectal cancer, hepatocellular cancer, lung cancer, pancreatic cancer, and osteosarcoma [31-36]. Survivin is also expressed in breast cancer [37]. The presence of survivin release in the stomach gland in this study suggests that heavy metals are caused by the influence of stomach gland cells.

V. CONCLUSİON

According to our study, consumption of carpet shell clam contaminated with heavy metal causes the production of survivin in cells. One of the causes of chronic inflammation of the stomach was found to be survivin production.

We think that consuming carpet shell clam that have been contaminated with heavy metal for a long period of time can cause more damage to the stomach.

Acknowledgements : We would like to express our appreciation to the Çanakkale Onsekiz Mart University Scientific Research Project Commission, which supported this study (ÇOMÜBAP, Project Number -2010/244).

Footnotes : At the time of this research, she was working at Department of Pathology of Çanakkale Onsekiz Mart University.

Ethics Statement : A total of 24 male Wistar albino rats, weighing 290–310 g, were used in the study. The study protocol was approved by the Çanakkale Onsekiz Mart University Ethics Committee for Animal Research.

REFERENCES

1. Balkwill F. Mantovani A. Inflammation and cancer: back to Virchow? *Lancet*, 357: 539-545, 2001.
2. Waisberg M, Joseph P, Hale B, Beyersmann D. Molecular and cellular mechanisms of cadmium carcinogenesis. Toxicology, 192: 95-117, 2003.
3. Hartwig A. Cadmium cancer Met Ions Life Sci, 491-507, 2013.
4. Timbrel JA. Biochemical mechanisms of toxicity: specific examples. Principles of Biochemical Toxicology, Taylor & Francis Ltd., London (1982)
5. Nieboer E, Tsuji LJ, Martin ID, Liberda EN. Human biomonitoring issues related to lead exposure. *Environ Sci Process Impacts*, 15: 1824-1829, 2013.
6. Clarkson TW. The three modern faces of mercury. *Environ Health Perspect*, 110: 11-23, 2002.
7. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature*, 454: 436-444, 2008.
8. Pesch B, Haerting J, Ranft U, Klimpel A, Oelschlägel B, Schill W. Occupational risk factors for renal cell carcinoma: agent-specific results from a case-control study in Germany MURC Study Group. Multicenter urothelial and renal cancer study. *Int J Epidemiol*, 29: 1014-1024, 2000.
9. Koedrith P, Kim H, Weon JI, Seo YR. Toxicogenomic approaches for understanding molecular mechanisms of heavy metal mutagenicity and carcinogenicity. *Int J Hyg Environ Health*, 216: 587-598, 2013.
10. Iannitti T, Capone S, Gatti A, Capitani F, Cetta F, Palmieri B. Intracellular heavy metal nanoparticle storage: progressive accumulation within lymph nodes with transformation from chronic inflammation to malignancy. *Int J Nanomedicine*, 5: 955-960, 2010.
11. Gezen MR, Demir N, Çetin M. Çanakkale Boğazı'nın Umurbey Kıyısındaki Deniz suyu ve Bazı Yumuşakçalarda (Bivalvia ve Gastropoda) Ağır Metal Düzeylerinin Araştırılması. *Ekoloji 2011*, 5-7 Mayıs, Düzce, 178, 2011.
12. Gezen MR, Demir N, Çetin M, Ballı M. Çanakkale Boğazı'ndaki Deniz Kestanesi (Paracentrotus lividus)' nin Ağır Metal Düzeyleri Üzerinde Bir Araştırma. *Ekoloji 2011*, 5-7 Mayıs, Düzce, 176, 2011.
13. Demir N, Gezen MR, Ballı M. Çanakkale Boğazı'nın Karacaören Kıyısındaki Deniz suyu ve Bazı Yumuşakçalarda (Bivalvia ve Gastropoda) Ağır Metal Düzeylerinin Araştırılması. *Ekoloji 2011*, 5-7 Mayıs, Düzce, 177, 2011.
14. Alteri DC. Validating survivinas a cancer therapeutic target. *Nat Rev Cancer*, 3: 46-54, 2003.
15. Ambrosini G, Adida C, Alteri DC. A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. *Nat Med*, 3: 917-921, 1997.
16. Gazouli M, Tzanakis N, Rallis G, Theodoropoulos G, Papaconstantinou I, Kostalis A, Anagnou NP, Nikiteas N. Survivin -31G/C promotor polymorphism and sporadic colorectal cancer. *Int J Colorectal Dis* 24: 145-150, 2009.
17. Junqueira LC, Carneiro J. (Ed: Solakoğlu C and Aytekin Y): Temel Histoloji. Nobel Tıp Kitapevleri, 290-295, 2009
18. Bakır K, Kapucuoğlu N, Bülbül HD. PCNA (Proliferating Cell Nuclerar Antigene) immunohistokimyasal boyanması ve agNORs'un meme karsinomunda prognozu belirlemedeki rolü. *Türk Patoloji Dergisi*, 12 (1): 35-38, 1996.
19. Avunduk MC, Tavlı Ş, Yol S, Tavlı L, Yavuz A, Güngör S, Yılmaz O. Mide karsinomlarında hücre proliferasyon belirleyicisi olarak PCNA, Ki 67 ve agNOR kullanımı. *Ankara Üniversitesi Tıp Fakültesi Mecmuası*, 53 (1): 11-15, 2000.
20. Cerulli N, Campanella L, Grossi R, Politi L, Scandurra R, Soda G. Determination of Cd, Cu Pb and Zn in neoplastic kidneys and in renal tissue of fetuses, newborns and corpses. *J Trace Elem Med Biol*, 20: 171-179, 2006.

21. Singh R, Gautam N, Mishra A, Gupta R. Heavy metals living systems: An overview. *Indian J Pharmacol*, 43: 246-253, 2011.
22. Aquino NB, Sevigny MB, Sabangan J, Louie MC. Role of cadmium and nickel in estrogen receptor signaling and breast cancer: Metalloestrogens or not? *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev* 30: 189-224, 2012.
23. Ambrosini G, Adida C, Sirugo G, Altieri DC. Induction of Apoptosis and Inhibition of Cell Proliferation by survivin Gene Targeting. *J Biol Chem* 273: 11177-11182, 1998;
24. Yazdani N, Sayahpour FA, Haghpanah V, Amiri P, Shahrabi-Farahani M, Moradi M, Mirmiran A, Khorsandi MT, Larijani B, Mostaan LV: Survivin gene polymorphism association with papillary thyroid carcinoma. *Pathol Res Pract* 15 (208): 100–103, 2012.
25. Upadhyay R, Khurana R, Kumar S, Ghoshal UC, Mittal B: Role of Survivin Gene Promoter Polymorphism (−31G>C) in Susceptibility and Survival of Esophageal Cancer in Northern India. *Annals of Surgical Oncology* 18 (3): 880-887, 2011.
26. Tran TJ, Master Z, Yu JL, Rak J, Dumont DJ, Kerbel RS. A role for survivin in chemoresistance of endothelial cells mediated by VEGF. *Proc Natl Acad Sci USA* 99: 4349-4354, 2002.
27. Rödel C, Haas J, Groth A, Grabenbauer GG, Sauer R, Rödel F. Spontaneous and radiation-induced apoptosis in colorectal carcinoma cells with different intrinsic radiosensitives: survivin as a radio resistance factor. *Int J Radiat Oncol Biol Phys* 55: 1341-1347, 2003.
28. Yamamoto T, Manome Y, Miyomoto A, Tanigawa N. Development of a novel gene therapy using survivin antisence expressing adenoviral vectors. *Gan To Kagaku Ryoho* 30: 1805- 1808, 2003.
29. Altieri DC. The molecular basis and potential role of survivin in cancer diagnosis and therapy. *Trends Mol Med* 7: 542-547, 2001.
30. Yamamoto T, Tanigawa N. The role of survivin as a new target of diagnosis and treatment in human cancer. *Med Electron Microsc* 34: 207- 212, 2001.
31. Wang YH, Chiou HY, Lin CT, Hsieh HY, Wu CC, Hsu CD, Shen CH. Association between survivin gene promotor -31 C/G polymorphism and urothelial carcinoma riskin Taiwanese population. Urology 73: 670-674, 2009.
32. Yang L, Zhu H, Zhou B, Gu H, Yan H, Tang N, Dong H, Sun Q, Cong R, Chen G, et.al. The association between the survivin C-31G polymorphism and gastric cancer risk in a Chinese population. *Dig Dis Sci* 54: 1021-1028, 2009.
33. Theodoropoulos GE, Michalopoulos NV, Panoussopoulos SG, Taka S, Gazouli M. Effects of caspase-9 and survivin gene polymorphisms in pancreatic cancer risk and tumor characteristics. *Pancreas* 39: 976-980, 2010.
34. Upadhyay R, Khurana R, Kumar S, Ghoshal UC, Mittal B. Role of survivin gene promoter polymorphism (-31G>C) in susceptibility and survival of esophageal cancer in northern India. *Ann Surg Oncol* 18: 880-887, 2011.
35. Borges Bdo N, Burbano RR, Harada ML. Survivin -31C/G polymorphism and gastric cancer risk in a Brazilian population. *Clin Exp Med* 11: 189-193, 2011.
36. Srivastava K, Srivastava A, Mittal B. Survivin promoter -31G/C (rs9904341) polymorphism and cancer susceptibility: a meta-analysis. *Mol Biol Rep* 39: 1509-1516, 2012.
37. Yamashita S, Masuda Y, Kurizaki T, Haga Y, Murayama T, Ikei S, Kamei M, Takeno S, Kawahara K. Survivin expression predicts early recurrence in early-stage breast cancer. *Anticancer Res* 27: 2803-8, 2007.

Aslı Muratl. (2018). Survivin Immunoreactivity in the Gastric Mucosa of Rats Feedind with Carpet Shell Clam (Ruditapes decussatus). Invention Journal of Research Technology in Engineering & Management (IJRTEM), 2(8), 23-31. Retrieved August 8, 2018, from www.ijrtem.com.